Morphological Study on a Case of Canine Hepatic Nodular Fibrosis

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ABSTRACT. Hepatic nodular fibrosis occurred in an 8-year-old male Papillon dog. Fibrous nodules, consisting of broad bands of collagen fibers, spine cells, and lipofuscin-laden foamy macrophages, were well-circumscribed and frequently linked up with the portal areas. Because the spine cells were positive for desmin and/or α-smooth muscle actin, they might be fio cells or myofibroblasts. These results suggest that both the spine cells and macrophages play an important role in the pathogenesis of hepatic nodular fibrosis, which might arise from the portal areas. — KEY WORDS: canine, liver, nodular fibrosis.

Hepatic nodular fibrosis in the dog is a very rare liver disease, as described in a male Chihuahua by Nakayama et al. [10]. The animal clinically showed an elevation in activities of GOT and GPT in the blood, and X-ray picture of focal calcification in the liver [10].

We describe here the results of morphological and immunohistochemical observations on a case of canine hepatic nodular fibrosis.

A dog, an 8-year-old male Papillon, was admitted to the Veterinary Teaching Hospital of Azabu University with marked emaciation, anorexia, vomiting, and excretion of yellowish urine. Blood test showed an elevation in enzyme activities of GOT (63 IU/L), GPT (100 IU/L), and ALP (more than 1,000 IU/L). Based on these findings, the clinical diagnosis was a hepatic neoplasm.

A laparotomy was performed to inspect the liver. The liver was hard and its surface was granular. The gallbladder adhered to the liver and was mildly dilated. There were innumerable granular nodules, pin-head or miliary in size which were diffusely distributed in the liver. The abdominal cavity showed serosanguineous ascites (about 100 ml). Occasionally adhesions between intestinal loops were present. There was also adhesion between the stomach and spleen which was slightly enlarged. Because of diffuse involvement of the liver, prognosis was judged to be poor. A portion of the lateral right lobe of the liver was excised for biopsy. The dog died of hepatic failure three days after the laparotomy, necropsy was not done. The biotin liver specimen was fixed in 10% neutral buffered formalin, processed routinely and embedded in paraffin. Three μm sections were cut and stained with hematoxylin and eosin (H.E.) and special stains including periodic acid-Schiff (PAS) reaction, Ziehl-Neelsen, orcein, Watanabe’s silver impregnation, and Masson’s trichrome. Frozen-sections were stained with Sudan III.

Immunohistochemical examinations were carried out on the paraffin sections using anti-lysozyme antibody (Dakopatts, Denmark), anti-α-smooth muscle actin (α-SMA) antibody (SIGMA CHEMICAL Co., U.S.A.), anti-human desmin antibody (DAKO A/S, Denmark), anti-α-SMA antibody (ADVANCE Co., Ltd., U.S.A.), anti-bovine type III collagen antibody (ADVANCE Co., Ltd., U.S.A.), anti-human fibronectin antibody (DAKO A/S, Denmark) and anti-human factor VIII-related antigen (FVIII-RAg) antibody (Dakopatts, Denmark). Localization of FVIII-RAg in the normal canine liver was also investigated. Lectin histochemistry was also done using Bauhinia purpurea lectin (BPA, E-Y Laboratories, Inc., U.S.A.). For electron microscopy, small blocks from the formalin fixed biotopic liver specimen were refixed in 1% osmium tetroxide buffered in phosphate, processed according to standard procedures and embedded in epoxy resin. Ultrathin sections were cut, stained with uranyl acetate and lead citrate, and examined using a Hitachi H-300 electron microscope at 75 kV.

Fibrous nodules in varying sizes were distributed irregularly throughout the liver specimen (Fig. 1). Some of them were fused and formed larger nodules. All the nodules were well-circumscribed and consisted of broad bands of collagen fibers, spine cells and pigment-laden foamy cells (Fig. 2). The centers of some nodules were calcified to various degrees (Fig. 3). The foamy cells showed no reactivity to anti-lysozyme antibody, but were bound with BPA. There also were pigment-laden foamy cell foci close by the fibrous nodules (Fig. 3). The cytoplasm of the foamy cells contained lipofuscin such as that stained with PAS, Ziehl-Neelsen, and showed stainability with Sudan III. The spine cells were frequently positive for α-SMA (Fig. 4a).

Fig. 1. Well-circumscribed fibrous nodules (asterisks) showing low cellularity. H.E. stain, x 105.
and occasionally reacted to the anti-desmin antibody (Fig. 4b).

Serial sections revealed that most of the fibrous nodules linked up with the portal areas (Fig. 5). Bile duct proliferation and fibrosis were frequently encountered in the portal areas without linkage to the fibrous nodules. Bile thrombi were seen throughout the liver.

On the other hand, pericellular (perihepatocellular) fibrosis occurred diffusely in the entire hepatic lobules (Fig. 6a) and was accompanied partially by elastin deposition (Fig. 6b). Type-III and -IV collagen and fibronectin were strongly positive in the space of Disse, outlining the sinusoids. Intense expression of desmin and α-SMA was observed in cells beside hepatocytes. Sinusoidal endothelial cells of the whole liver specimen frequently expressed FVIII-RAg (Fig. 7). In the normal canine liver, FVIII-RAg was observed in the endothelium of the portal veins, arterioles and central veins, but it was not found in the sinusoidal endothelium.

Electron microscopically, collagen fibrils and basement membrane-like substances were seen in spaces between hepatocytes and sinusoidal endothelial cells containing many lysosomes (Fig. 8: inset and Fig. 9). The foamy cells had a lobulated nucleus and abundant secondary lysosomes filled with variously electron-dense substances.

It has been demonstrated that Ito cells are a major producer of collagen in the normal and damaged liver [2]. Human Ito cells contain α-SMA protein [15] of which expression is enhanced in several diseased conditions [13]. Recent in vitro studies revealed that Ito cells are motile and contractile [5], and furthermore, Ito cells in culture transform into myofibroblasts containing α-SMA protein [14]. Ito cells of dogs show a positive reactivity to desmin [8]. Myofibroblasts also express various proteins of intermediate
filaments such as desmin [11]. In the present case, the spindle cells expressing α-SMA and/or desmin in the nodules might be Ito cells or myofibroblasts and play an important role in fibrogenesis in the nodules.

The pigment (lipofuscin)-laden foamy cells were located in the nodules, suggesting that these cells might be related to the fibrogenesis, as pointed out by Nakayama et al. [10]. The foamy cells are considered to be macrophages because of their ultrastructural features and positive reactivity to BPA, which is also a marker for Kupffer cell [9].

A recent review of hepatic fibrosis has emphasized significance of a variety of cytokines, such as transforming growth factor-β and platelet-derived growth factor in fibrogenesis [6]. Kupffer cell-derived cytokines stimulate collagen production of Ito cells in vitro [1, 7]. Paracrine and/or autocrine mechanisms between Ito cells/myofibroblasts expressing desmin or α-SMA and foamy macrophages might be involved in pathogenesis of the fibrous nodules as seen in the present case.

FVIII-RAg is not expressed in the sinusoidal endothelium.
in the normal liver of humans [4] and dogs. In the present case, FVIII-RAg was occasionally expressed in the sinusoidal endothelium of the liver.

This phenotypic alteration of the endothelium and formation of the basement membranes in the space of Disse may indicate sinusoidal capillarization [12] such as that seen in the cirrhotic liver [12] and hepatocellular carcinoma [3]. However, the relation of the fibrous nodules to the sinusoidal capillarization and the pericellular fibrosis is unclear in the present study.

Most of the fibrous nodules linked up with the portal areas in the present case. Bile duct proliferation and fibrosis were encountered in the portal areas without linkage to fibrous nodules. These findings indicate that the fibrous nodules might develop from the portal areas.

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REFERENCES